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Changes in Molecular Size of Previously Deposited and Newly Synthesized Pea Cell Wall Matrix Polysaccharides[1]

Effects of Auxin and Turgor

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ABSTRACT

Effects of indoleacetic acid (IAA) and of turgor changes on the apparent molecular mass (M_r) distributions of cell wall matrix polysaccharides from etiolated pea (*Pisum sativum* L.) epicotyl segments were determined by gel filtration chromatography. IAA causes a two- to threefold decline in the peak M_r of xyloglucan, relative to minus-auxin controls, to occur within 0.5 hour. IAA causes an even larger decrease in the peak M_r concurrently biosynthesized xyloglucan, as determined by [^{3}H]fucose labeling, but this effect begins only after 1 hour. In contrast, IAA does not appreciably affect the M_r distributions of pectic polyuronides or hemicellulosic arabinose/galactose polysaccharides within 1.5 hours. However, after epicotyl segments are cut, their peak polyuronide M_r increases and later decreases, possibly as part of a wound response. Xyloglucan also undergoes IAA-independent changes in its M_r distribution after cutting segments. In addition, the peak M_r of newly deposited xyloglucan increases from about 9 kilodaltons shortly after deposition to about 30 kilodaltons within 0.5 hour. This may represent a process of integration into the cell wall. A step increase in turgor causes the peak M_r of previously deposited xyloglucan (but not of the other major polymers) to increase about 10-fold within 0.5 hour, returning to its initial value by 1.5 hours. This upshift may comprise a feedback mechanism that decreases wall extensibility when the rate of wall extension suddenly increases. IAA-induced reduction of xyloglucan M_r might cause wall loosening that leads to cell enlargement, as has been suggested previously, but the lack of a simple relation between xyloglucan M_r and elongation rate indicates that loosening must also involve other wall factors, one of which might be the deposition of new xyloglucan of much smaller size. Although the M_r shifts in polyuronides may represent changes in noncovalent association, and for xyloglucan this cannot be completely excluded, xyloglucan seems to participate in a dynamic process that can both decrease and increase its chain length, possible mechanisms for which are suggested.

Auxin-induced enlargement of a plant cell results from a "loosening," or increase in irreversible extensibility of its cell wall, which permits the wall to be deformed by the cell's turgor pressure. The nature of wall loosening, at the level of wall structure, continues to be debated. Many workers have expected, based on the popular sycamore primary wall structural model (2), that growing walls would be loosened by breaking covalent cross-links between wall polymers. However, our study of pea primary walls (50) revealed that its major polysaccharide components are mostly not covalently coupled. An alternative possible loosening mechanism, breakdown of polymer backbones, has received experimental support. Auxin-induced breakdown or turnover of hemicellulosic glucans has been reported in both monocots and dicots (18, 23, 33, 35). Estimations of wall polysaccharide molecular mass by GFC[3] have indicated that auxin can cause the M_r of XG to shift toward lower values (24, 26, 38, 42, 44, 53). Auxin-induced release of soluble XG from the wall (33, 34, 51) also suggests that XG is being altered.

In the present work, the M_r distributions of etiolated pea epicotyl pectic and hemicellulosic polysaccharides were compared using GFC during treatment with or without growth-inducing concentrations of IAA and after step increases in turgor pressure, which were found to cause changes at least as dramatic as those due to IAA. In labeling experiments, the M_r distributions of newly synthesized wall polysaccharides, and the effect of IAA thereon, were also determined.

MATERIALS AND METHODS

Growth and Preparation of Tissue

All experiments were performed using 8-mm segments cut from the third internode of 7-d-old pea seedlings, *Pisum sativum* L. cv Alaska, prepared as described previously (50). Segments were depleted of endogenous auxin by a 2-h incubation at 35°C, either in a 100% RH chamber (air pretreatment) or floated in a 10 mM potassium phosphate buffer, pH 7.0 (buffer pretreatment). Segments were then incubated for various periods at 25°C in a treatment medium consisting of

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[3] Abbreviations: GFC, gel filtration chromatography; M_r, molecular mass relative to dextran standards; XG, xyloglucan; FC, fusicoccin; AG, arabinose/galactose polysaccharides (arabinogalactan, arabinan plus galactan).

the same buffer with or without 17 μM IAA or 1 μM FC before wall isolation.

Cell Wall Isolation and Fractionation

Walls from treated segments were isolated and chemically fractionated into pectic (ammonium oxalate-soluble), hemicellulosic (KOH-soluble), and cellulosic fractions as described previously (50). GFC was also conducted and calibrated, and column fractions were analyzed, as described previously (50).

Radioisotope Experiments

The cuticle of each internode segment was abraded by gently rubbing with a thick aqueous suspension of No. 305 emery powder (Edmund Scientific, Barrington, NJ). Following air pretreatment as above, 60 segments were incubated in 10 mL of the treatment medium given above but containing also either 5 μCi of [U-^{14}C]glucose or 30 μCi of [5,6-^{3}H] fucose. Radiolabel content of polymer fractions was determined by liquid scintillation, using a Delta 300 liquid scintillation counter (TM Analytic) and ACS liquid scintillation cocktail (Amersham). Counting efficiency was about 20% for ^{3}H and 60% for ^{14}C.

RESULTS

To assess M_r changes in pea cell wall matrix polysaccharides during elongation, we initially used third internode segments that had been kept 2 h in moist air after cutting (air pretreated), similar to the material used in studying IAA enhancement of polysaccharide synthase activities (48). In such segments, large changes in size distributions occurred, which proved to be compounded of time-dependent, IAA-dependent, and turgor-dependent effects. We also examined segments pretreated by keeping them 2 h in buffer (buffer pretreated), so that a turgor step-up would not occur when incubation in buffer with or without IAA was begun. Elongation of air- as well as of buffer-pretreated segments in response to IAA (data not shown) was very similar to elongation records in the literature (32, 33).

Pectic Polyuronides

The polyuronides of freshly cut internode segments give a relatively symmetrical GFC peak centered at about 850 kD; during air pretreatment this peak shifts upward to around 1500 kD (Fig. 1a). After about 1 h of subsequent incubation in buffer, the peak begins to shift back downward, returning to about the same value as in freshly cut segments by 1.5 h (Fig. 1, b–d).

An at least partly comparable polyuronide M_r upshift also occurs during buffer pretreatment, as shown by the control profile in Figure 2. However, that profile is considerably broader than most of those in Figure 1 and has a shoulder in the M_r range of the initial (freshly cut tissue) peak, suggesting either an incomplete upshift or a partial return to the initial

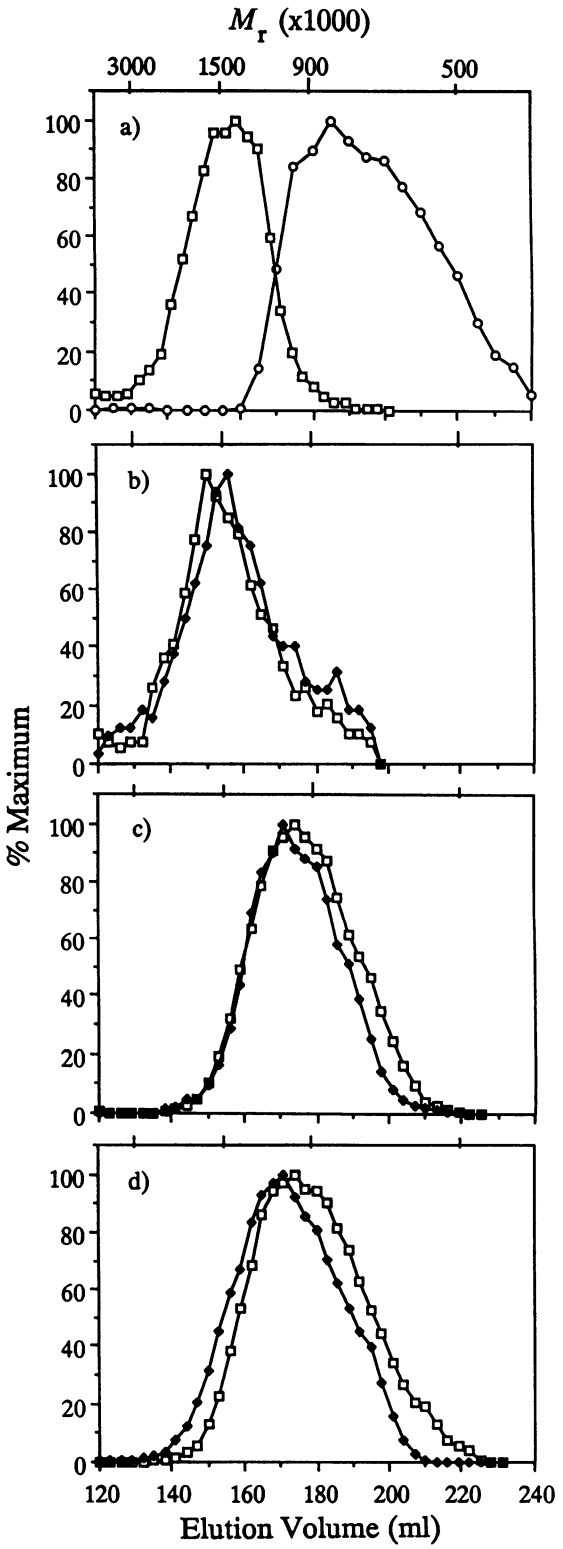

Figure 1. Pectic polyuronide M_r distributions from (a; ○) freshly harvested segments and from air-pretreated segments at various times after the start of incubation in buffer without (□) or with (♦) 17 μM IAA: a, 0 h; b, 0.5 h; c, 1.0 h; d, 1.5 h.

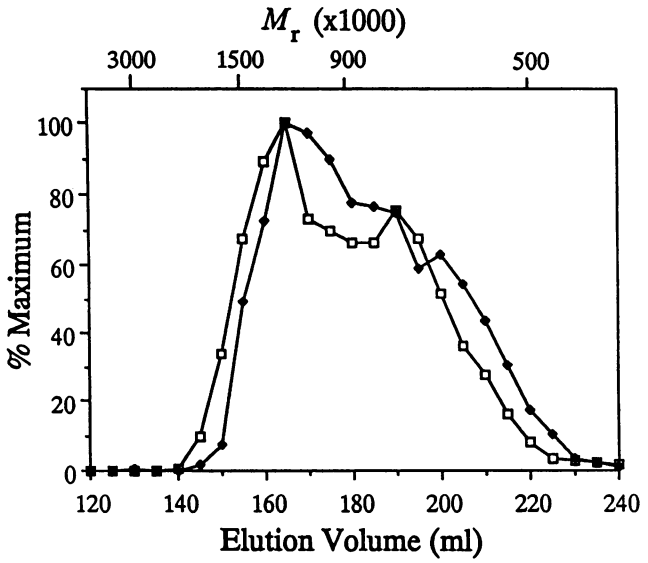

Figure 2. Pectic polyuronide M_r distribution from buffer-pretreated segments incubated 0.5 h with (♦) or without (□) IAA.

M_r under these conditions at the time represented by Figure 2 (a time equivalent to that in Fig. 1c).

These experiments reveal virtually no effect of IAA on the changes in polyuronide M_r distribution, at any time between 0.5 and 1.5 h of treatment (Figs. 1 and 2). In most cases, the plus-IAA peak was slightly upshifted relative to its control, but we doubt this effect is significant. Treatment with the auxin-mimetic toxin FC for 1.5 h gave a polyuronide M_r distribution identical with that for the IAA treatment in Figure 1d (data not shown).

Hemicelluloses

As previously described (50), pea hemicelluloses can be resolved by GFC into a high M_r peak comprising mainly AG, plus a lower M_r peak of XG. The AG's M_r distribution does not change significantly during pretreatment, or during incubation with or without IAA (data not shown). The XG component, on the other hand, undergoes complex changes.

The GFC profile of XG from fresh tissue peaks at 30 kD, with a minor component at 300 kD (Fig. 3a). Either air or buffer pretreatment leads to loss of this higher M_r component, plus a modest downshift of the main peak to 20 to 25 kD (Fig. 3a). Incubation of air-pretreated segments for just 0.5 h in buffer without IAA causes the profile to shift up, transiently, to peak at about 300 kD (Fig. 3b), returning virtually to its initial position by 1.5 h after the start of buffer incubation (Fig. 3, c and d). A somewhat comparable upshift in the XG peak, in this case from 30 to about 200 kD, occurs if segments that have been pretreated 2 h in 0.2 M mannitol are incubated 0.5 h in buffer (Fig. 4).

Including IAA in the incubation buffer completely prevents the initial upshift of the XG peak just described (Fig. 3, b and c). When the minus-IAA control's XG peak shifts back down,

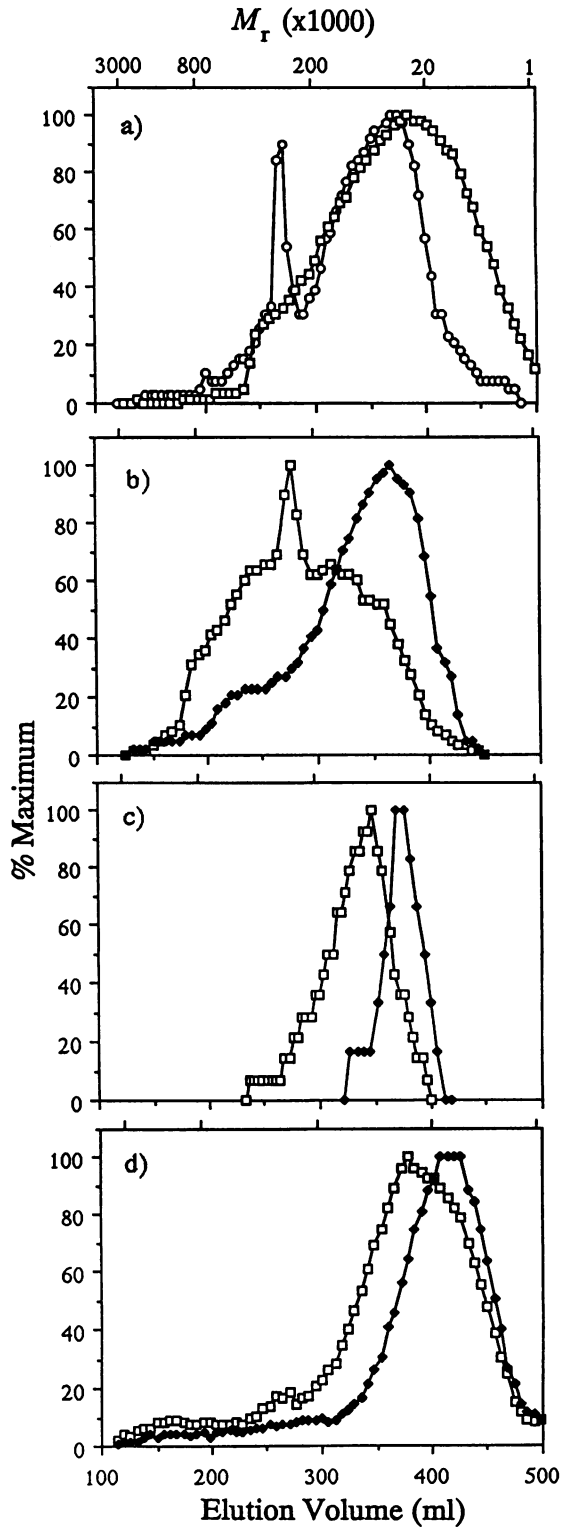

Figure 3. XG M_r distribution from (a; ○) freshly harvested segments and from air-pretreated segments at various times after the start of incubation in buffer without (□) or with (♦) IAA: a, 0 h; b, 0.5 h; c, 1.0 h; d, 1.5 h.

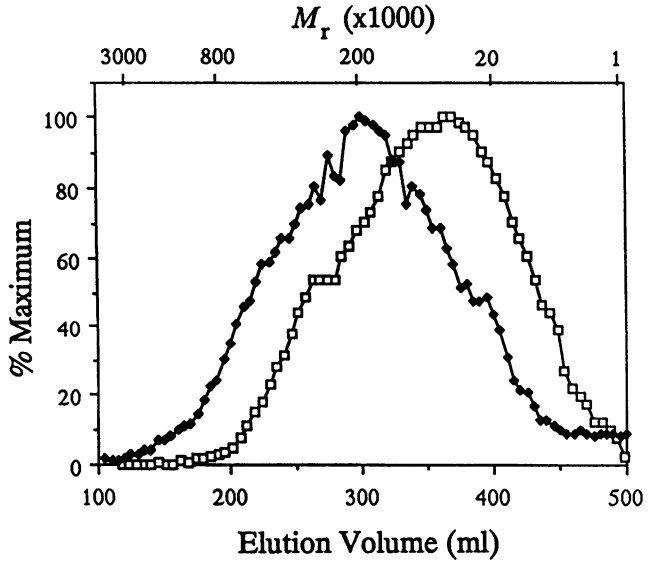

Figure 4. XG M_r profiles from segments pretreated 3 h in 0.2 M mannitol (□) and from mannitol-pretreated segments incubated 0.5 h in mannitol-free buffer without IAA (♦).

the IAA-treated tissue's peak also declines, down to about 10 kD by 1.5 h (Fig. 3d), thus remaining substantially lower than the control throughout the period.

XG from buffer-pretreated segments peaks at about the same position as with air-pretreated tissue (Fig. 5, control profile). Although the XG profiles in Figure 5 are rather narrower than in Figures 3 and 4, a broader peak, more comparable to the latter figures, was obtained in other experiments on similarly pretreated tissue (*e.g.* Fig. 6, control profile). IAA treatment of buffer-pretreated segments causes a marked downshift of the XG peak, within 0.5 h, to about 10 kD (Fig. 5).

The monosaccharide composition (determined as in ref. 50) of the upshifted XG peak from buffer incubation of air-pretreated segments, and of the downshifted XG peak from IAA treatment, does not differ appreciably from that given in table III of ref. 50 for the typical pea XG peak of approximately 25 kD (data not shown).

Contrary to our expectation, FC does not cause a decline in XG M_r relative to the control, as does IAA. We observed this with both buffer-pretreated (Fig. 6) and air-pretreated (not shown) tissue. Instead, in both of these experiments the XG from FC-treated tissue showed a peak M_r considerably higher than that of the control.

Newly Synthesized Wall Polysaccharides

M_r distributions of newly synthesized and deposited polymers were determined by GFC of wall extracts from air-pretreated pea segments that had been briefly fed labeled glucose, which is rapidly converted into all wall polymer monosaccharide components and incorporated into the polymers being deposited (49). After only 10 min exposure of tissue to [^{14}C]glucose, the pectin fraction gives a single peak of ^{14}C centered at about 1000 kD, closely resembling that of the total polyuronides in the pectin extract (Fig. 7a). The pulse-labeled hemicellulose fraction, on the other hand, gives two peaks of ^{14}C (Fig. 7b). That at about 1000 kD corresponds with the AG peak in the GFC profile of whole-wall hemicelluloses (50). The ^{14}C peak at about 9 kD presumably represents the hemicellulose's XG component, which apparently has a peak M_r only about one-third that of the wall's bulk XG. ^{14}C incorporation into XG in Figure 7b is disproportionately large relative to that in the AG peak, compared with the wall's bulk content of these polymers (Fig. 4 of ref. 50). This is because in a short [^{14}C]glucose-feeding period such as that used here, much greater isotope dilution prevails in nonglucose sugars than in glucose (49). Because glucose is the major component of the XG peak but only a minor component of the AG peak (50), early incorporation is biased in favor of the XG peak. In agreement with this interpretation, after feeding glucose for 2 h (data not shown), a period several times that needed to give isotope equilibrium in the polysaccharide synthesis pathway (1), the relative incorporation into the XG and AG peaks shifts to resemble closely the relative amounts of total sugar in these respective peaks, as illustrated in figure 4 of ref. 50.

Changes in XG size distribution after pulse-labeling periods >10 min were studied by feeding [^{3}H]fucose, because XG contains fucose and exogenous fucose is incorporated into XG during biosynthesis but is not converted significantly into other monosaccharides (7, 14). As expected on the basis that XG is the principal fucose-containing dicot wall polymer, after [^{3}H]fucose feeding we found about 93% of the wall-incorporated ^{3}H in the hemicellulose fraction (the remainder in pectin), and this ^{3}H occurs only in fucose (data not shown). Furthermore, peak ^{3}H incorporation occurs in the relatively low M_r part of the hemicellulose GFC profile (Fig. 8a), where

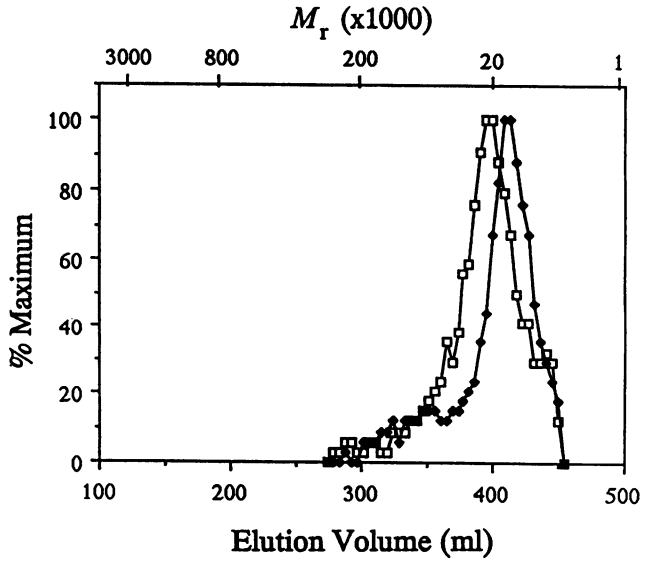

Figure 5. XG M_r distribution from buffer-pretreated segments incubated for 0.5 h with (♦) or without (□) IAA.

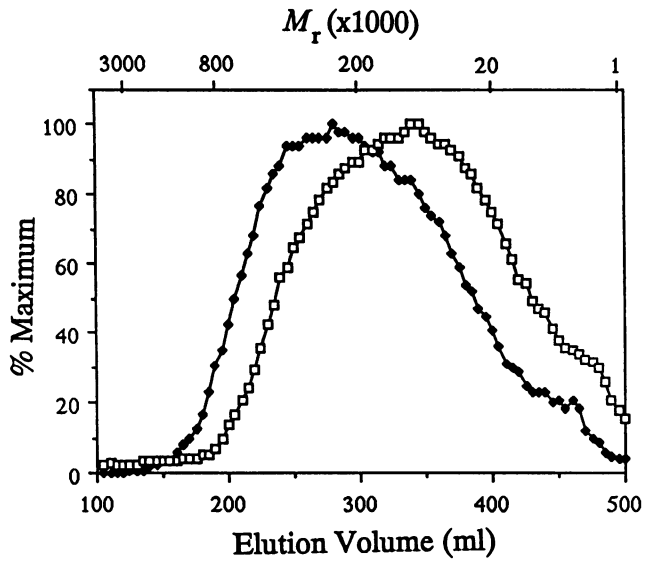

Figure 6. XG M_r distribution from buffer-pretreated segments incubated 1.25 h with (♦) or without (□) 1 μM FC.

the principal peak of bulk XG typically occurs (50). This ^3H peak is presumed, in what follows, to represent newly synthesized XG. The small secondary ^3H peak in the high-M_r (AG) portion of the profile (Fig. 8) may represent fucose incorporated into the small amounts of XG and of rhamnogalacturonan that occur in that part of the GFC profile (50).

After 30 min, which was the shortest practicable [^3H]fucose-labeling period, the peak of newly synthesized XG occurs at about 20 kD (Fig. 8a), in contrast to the 9 kD peak seen after a 10-min [^{14}C]glucose pulse (Fig. 7b). At 30 min the new XG's peak M_r does not differ between IAA-treated and control tissue (Fig. 8a), even though the bulk wall XG peak M_r values differ greatly between these treatments at that time (Fig. 3b). Because in these experiments air-pretreated tissue was used, the newly synthesized XG peak corresponds in M_r with the bulk XG of IAA-treated tissue and not of the control, whose XG M_r is considerably upshifted at this time (Fig. 3b).

After 60 min of [^3H]fucose feeding, control and plus-IAA XG profiles are still similar, but the IAA profile has broadened and started to shift downward toward lower M_r values (Fig. 8b). By 90 min (Fig. 8c), the [^3H]XG peak from IAA-treated tissue has declined to about 5 kD, a considerably lower M_r than that of the wall's bulk XG at this time (Fig. 3d). The control tissue's [^3H]XG peak, in contrast, remains unshifted and now corresponds closely with the tissue's bulk XG peak M_r. Similar results (not shown) were obtained after 120 min of [^3H]fucose feeding.

The amount of ^3H in the entire XG peak was compared with that in polymers between 20 and 60 kD after different times of [^3H]fucose feeding (Table I). ^3H incorporation into XG in minus-IAA tissue continues (as expected) throughout the feeding period. However, in IAA-treated segments ^3H in the 20 to 60 kD region, where the original ^3H peak was located, declines markedly from 60 to 90 min, whereas total ^3H in XG (right column of Table I) levels out or may also decline somewhat.

Treatment of emery-abraded segments with citrate buffer, pH 4.5, for 1 h during [^3H]fucose feeding does not shift the peak M_r away from about 20 kD (Fig. 9). The low pH treatment actually caused a slight upshift of the ^3H peak which was less pronounced than the effect of FC (Fig. 6).

DISCUSSION

The results consistently reveal severalfold downshifts in peak M_r of XG, but not of AG or pectic polyuronides, after exposure of pea internode segments to IAA. The XG downshift agrees with previous reports on azuki bean epicotyl (24, 42, 44), oat coleoptile (26), and cucumber (53) or pine (38) hypocotyl segments. However, the cited previous work mostly reported very broad, flat XG M_r profiles that were shifted only slightly by IAA relative to their breadth, corresponding to only about 10 to 30% reduction in calculated weight-average M_r. Practical experience with polysaccharide GFC makes one skeptical of inferences based on such small shifts.

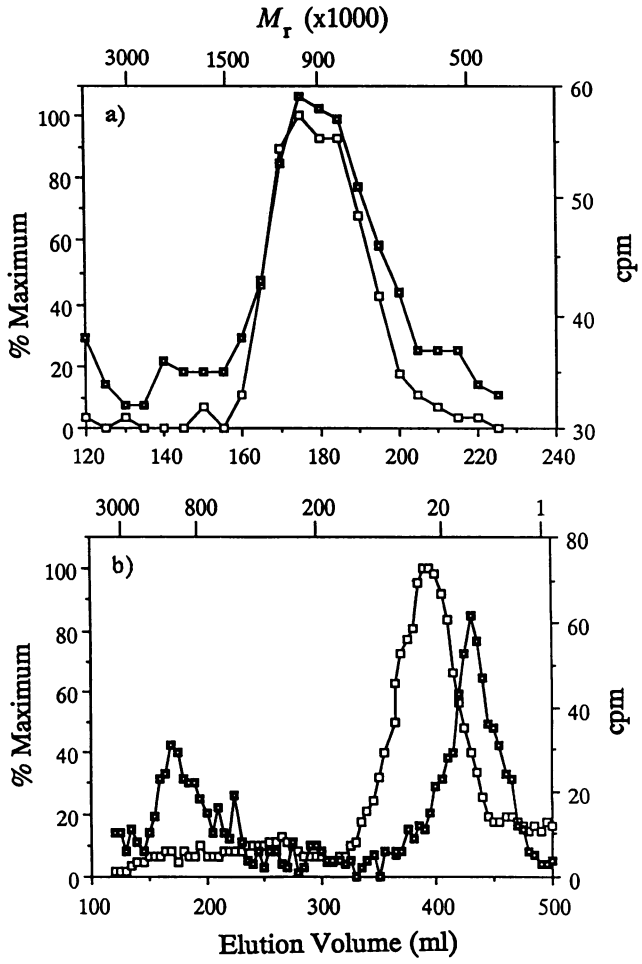

Figure 7. M_r distributions after 10-min labeling of air-pretreated segments with [^{14}C]glucose in the presence of IAA. a: Pectin fraction ■, ^{14}C; □, uronic acid by colorimetric assay. b: Hemicellulose fraction ■, ^{14}C; □, XG by colorimetric assay.

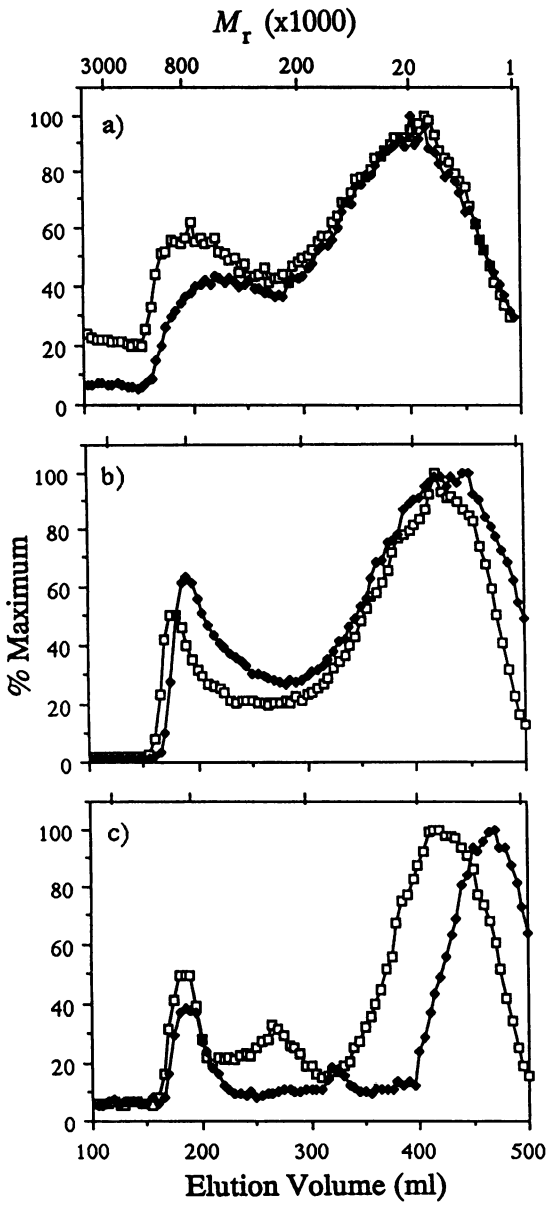

Figure 8. M_r distribution of ^{3}H-labeled hemicelluloses (principally XG) from air-pretreated segments after incubation with [^{3}H]fucose without (□) or with (♦) IAA for: a, 0.5 h; b, 1.0 h; or c, 1.5 h.

Table I. *[^{3}H]Fucose Incorporation into XG*

Data are from the experiment of Figure 8. Results have been corrected for cpm that fell beyond the 500 mL collection limit, in the +IAA profiles of Figure 8, b and c, by extrapolation assuming a symmetrical peak.

Time	Incorporation into			
	20–60 kD		2–200 kD	
	−IAA	+IAA	−IAA	+IAA
h	*cpm*			
0.5	5,330	7,350	14,000	19,140
1.0	19,280	26,770	57,100	89,880
1.5	27,740	5,870	89,490	82,620

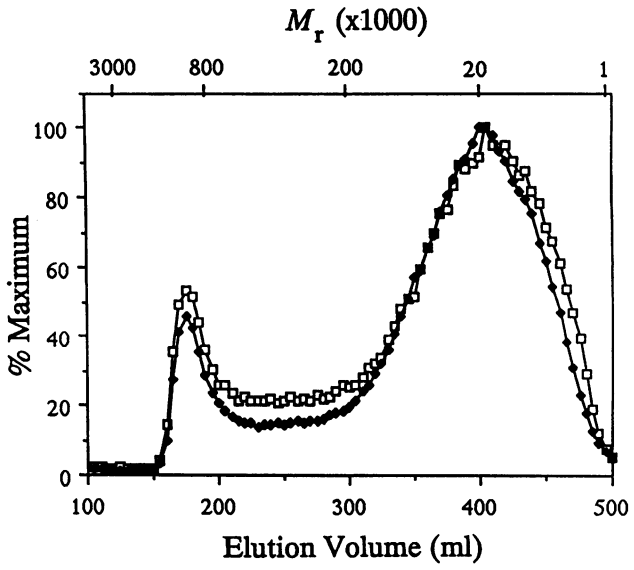

Figure 9. Effect of low pH on M_r distribution of ^{3}H-labeled hemicelluloses. Abraded, air-pretreated segments were incubated 1.0 h with [^{3}H]fucose in 10 mM K citrate buffer, pH 4.5 (♦), or K phosphate buffer, pH 7.0 (□).

In contrast, in the present results, IAA displaces a relatively sharp XG peak by a substantial fraction of its width, corresponding to a severalfold reduction in peak M_r. Thus, we believe that these results demonstrate IAA-induced XG M_r downshifts much more convincingly.

Earlier work on pea, using pulse-chase labeling to measure turnover of wall polymers (18, 33), indicated that IAA induces some disappearance of XG from the cell wall, with the appearance of comparable small amounts of soluble XG, apparently released from the wall. The present results show that IAA action on wall XG is much more extensive than the turnover data suggested. Those data indicated that IAA-independent turnover of galactan greatly exceeds the IAA-stimulated disappearance of XG. The relatively narrow AG M_r distribution (50) and lack of temporal changes suggestive of degradation (present results) may mean that galactan turnover occurs by completely removing, from the wall, those galactan chains that are attacked. In contrast, IAA action appears to reduce the size of most or all of the XG molecules in the wall, while removing only a few of them and completely degrading even fewer, thus with little actual turnover. This difference agrees with findings on pea cell wall autolytic activity, which was reported to involve primarily breakdown of (arabino)galactan to monosaccharides, with relatively minor release of xylose and glucose, virtually all in polymeric form (11). Thus, the XG M_r downshift caused by IAA might be assumed (as in most of the cited previous reports of XG M_r changes) to represent endohydrolytic cleavage of XG chains.

However, the present results show that the IAA-induced XG M_r downshift in pea takes place against a background of time-dependent changes in XG M_r in the minus-IAA controls, notably a dramatic but transient M_r upshift that occurs when air-pretreated segments are immersed in aqueous incubation

medium. Under these conditions, the initial IAA effect is not an actual M_r downshift but an inhibition of the mentioned upshift. A somewhat similar IAA inhibition was previously reported for pine hypocotyl segments (38). Our short-term labeling experiments also revealed an upshift in M_r that occurs between 10 and 30 min after newly synthesized XG is deposited in the wall. Furthermore, we observed an IAA-independent M_r increase in pectic polyuronides during the first 2 h after cutting epicotyl segments, followed later by a decrease. Because at first sight it might seem biologically and biochemically implausible that the chain length of previously deposited wall polymers could substantially increase, we need to consider whether the observed M_r increases, and indeed possibly even the decreases, might represent something other than changes in the length of previously formed polysaccharide chains.

Nature of M_r Changes

The following are possible explanations (other than changes in polysaccharide chain length) of the observed M_r changes, that need to be considered.

Synthesis and Deposition of New Polymers of Different M_r

Under the conditions used in the present experiments, pea stem segments deposit new wall material at a net rate of only about 1.5%/h (1). Radioisotope experiments show that XG turnover occurs at only a very small fraction of the rate of XG synthesis and that polyuronide turnover is negligible (33). Therefore, the observed large shifts in M_r of pectin within 2 h and of bulk XG within 1 h cannot be due to a change in the M_r of newly synthesized polymers. For XG the [^{3}H]fucose incorporation experiments confirm this, by showing that newly synthesized XG does not participate in the above-mentioned M_r upshift and is not downshifted by IAA until later than 1 h. Moreover, the amount of ^{3}H in 20 to 60 kD XG dramatically decreases between 1 and 1.5 h of IAA treatment (Table I). This indicates that IAA causes many of the previously synthesized XG molecules in this size range to disappear. Furthermore, ^{3}H in XG as a whole (right column in Table I) also apparently decreases during this period, despite the continued availability of [^{3}H]fucose to the cells and its continued incorporation in the minus-IAA controls. This suggests that IAA causes a net removal of previously synthesized XG from the wall in the period after 1 h.

Covalent Cross-Linking between Polymer Chains

The monosaccharide composition of the XG peak does not change during the observed M_r adjustments, indicating that they are not caused by coupling to (or decoupling from) other wall polymers such as AG. Coupling of AG to pectic polyuronide in large enough amount to yield the observed increase in polyuronide M_r would largely deplete the hemicellulosic AG peak, which did not happen. Coupling of polysaccharides to wall protein(s), as was inferred to take place in fungal cell walls (12), is unlikely to explain the observed M_r increases, because in our procedure (50) the cell walls were treated extensively with protease before polysaccharide extraction; neither pectin nor XG are known to be linked, to any substantial extent, to protease-resistant proteins such as hydroxyproline-rich wall proteins. Changes in the extent of postulated cross-linking of pectic polymers by ester bonds (15) might explain changes in polyuronide M_r but cannot account for changes in XG because any ester bonds would have been hydrolyzed by the alkali used to extract XG from the cell wall (50).

Changes in Noncovalent Association between Polysaccharide Chains

Because the polyuronide GFC profile may be influenced by noncovalent aggregation (50), pectic M_r changes may be due to changes in tendency toward association, which might result, for example, from changes in methyl ester content (*e.g.* pectin methylesterase action). XG, on the other hand, showed no tendency toward aggregation when subjected to the conditions under which we observed pea polyuronides to aggregate *in vitro* (50). Nevertheless, if xylose side groups were removed from XG by xylosidase action, the chains might conceivably associate. However, the M_r-upshifted XG peak showed no appreciable decrease in xylose content. Removal of XG acetyl groups (29) might also promote association, but because any esters, as noted above, would have been hydrolyzed during alkali extraction of XG, they could not be responsible for the observed M_r differences. XG molecules might associate as a consequence of their tendency to chelate heavy metals (30). This possible effect cannot strictly be excluded, but why it would occur only after certain tissue treatments is not clear.

In conclusion, changes in ester cross-linking, and/or in noncovalent association, may explain the observed changes in pectin M_r, and changes in association are not altogether ruled out for XG. It seems probable, however, that the decreases in XG M_r involve a decrease in XG chain length and seems possible that at least some of the observed M_r increases represent an actual increase in XG chain length. In this event, the chain length of cell wall XG would be in a dynamic state, subject to both diminution and extension. Possible mechanisms for these changes will now be considered.

Possible Mechanisms for XG M_r Changes

Decrease in Chain Length

XG-degrading enzymes that occur in cell walls can cause an M_r downshift (31) that resembles the *in vivo* effect of IAA on XG described here. Pea tissue contains cellulase (endo-β-1,4-glucanase), which is capable of hydrolyzing XG (20). Because IAA induces cellulase in pea (13), it has repeatedly been suggested that cellulase is responsible for IAA-induced XG breakdown (16, 17, 20, 41). However, induction of cellulase by IAA occurs only very slowly (52) compared to the

XG M_r response observed here, and substantial induction requires much higher IAA concentrations than were used in the present work, concentrations that inhibit rather than stimulate cell elongation (10). Therefore, the much-studied IAA induction of cellulase seems unable to explain the rapid downshifting of XG M_r by elongation-inducing concentrations of IAA. Alternative mechanisms might be stimulation, by IAA, of cellulase or xyloglucanase secretion from the cell into the wall or activation of preexisting wall-localized cellulase or xyloglucanase by IAA, for example by IAA-induced H^+ extrusion (16). But pea cellulase is reported to have a pH optimum of 5.5 to 6.0 (6, 39), whereas IAA-induced H^+ extrusion lowers pea cell wall pH to about 5.0, from a control value of about 6.0 (28), thus apparently tending to inhibit rather than activate this enzyme. Furthermore, according to our results neither low external pH nor the H^+ extrusion-inducing toxin FC (40) causes a downshift in pea XG M_r. These latter results may have a special explanation (see below). But still another problem is that wall-localized pea cellulase is reportedly confined to the inner surface of the wall (3), so apparently does not have access to most of the wall's XG, as it must to account for the present observations. A distinct xyloglucanase, regulated differently than is known for pea cellulase, is an alternative possible mechanism. Hoson (22) recently reported that treating azuki bean tissue with IAA for just 3 h caused some enhancement of a wall-bound autolytic activity that releases glucose and xylose, probably from XG.

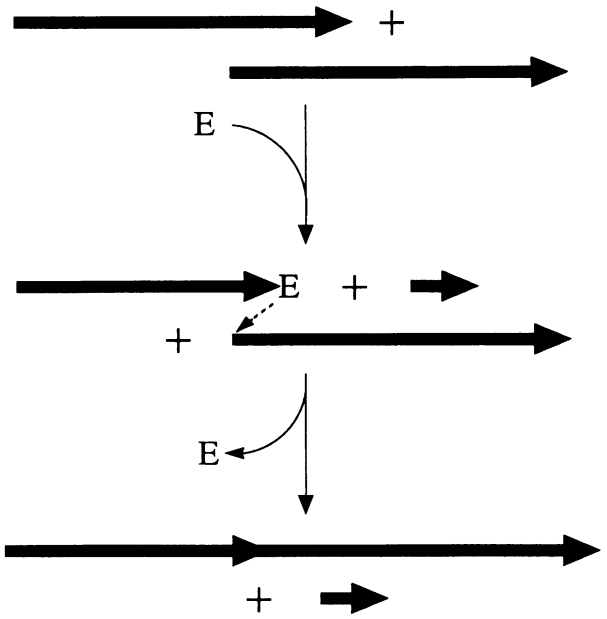

Figure 10. Possible transglycosylase reaction to increase the M_r of XG chains in the cell wall. E, transglycosylase; heavy arrows, XG chains, the arrowhead being the reducing end of each; buried arrowhead, former reducing end, after coupling to another XG chain through transglycosylase action. Additional transglycosylase steps involving other XG chains could increase the M_r still further. The small M_r XG fragments formed as a side product of the reaction would presumably be soluble and thus not detected as part of the cell wall.

Increase in Chain Length

Elongation of previously deposited XG chains by synthase action (addition of sugar residues from sugar nucleotide glycosyl donors) seems very unlikely, because it would require that sugar nucleotides be released from the cell into the cell wall and because synthase activity is not known to occur in cell walls. Alternatively, transglycosylase action (Fig. 10) could split an XG chain and transfer the former nonreducing half to another XG chain. If the enzyme attacked XG chains near their reducing ends and/or acted repetitively on the reducing-end fragment from the first transglycosylation, XG chain length would increase without accumulation of smaller M_r fragments other than soluble oligosaccharides or sugars, which we would not have detected in our procedure. Somewhat analogous transglycosylation reactions occur in fructan and amylopectin synthesis and have been suggested for cell wall polysaccharides; indications of a transglycosylation reaction involving XG were reported (5).

Because some glycoside hydrolases also have transglycosylase activity, diminution and extension of XG chain length could be due to one and the same enzyme. Whether or not this is so, shifts in M_r would be caused by changes in the balance between hydrolytic cleavage and transglycosylative extension of XG chains. These respective actions may well be subject to separate controls, leading to the possibility of somewhat complex responses to experimental treatments, as observed in the present work.

Significance of IAA-Independent Changes in M_r

The pectic polyuronide M_r distribution undergoes, as noted above, a substantial upshift followed by a gradual decline to about its initial position, over a period of several hours after pea internode segments are cut (Fig. 1). In azuki bean segments, Nishitani and Masuda (42) observed a possibly similar progressive increase in pectin M_r. These changes may be part of a wound response provoked by cutting the segments.

The pea XG M_r profile also changes with time after cutting epicotyl segments, the secondary peak at about 300 kD largely disappearing and becoming replaced by a tail of material on the low-M_r side of the principal (approximately 20–30 kD) XG peak (Fig. 3a). This, like the polyuronide changes just discussed, may be part of a wound response. The XG profile undergoes two other apparently IAA-independent changes, previously mentioned. The increase in peak M_r from about 9 to about 20 kD that occurs during the first 30 min after deposition of newly synthesized XG into the cell wall (Figs. 7b and 8) may represent a process by which new XG molecules become integrated into the wall structure. This might take place by transglycosylation, as noted above, perhaps involving the same enzymatic mechanism as is responsible for the reported coupling of XG-derived oligosaccharides to extracellular polysaccharides (5). Our results give no indication of a comparable integration mechanism for AG or pectic polyuronides, which in contrast to XG exhibit essentially their final M_r distribution within minutes of their export into the wall.

The transient XG peak M_r upshift from about 30 to about 300 kD during the first 0.5 h after air-pretreated segments are placed in incubation buffer (Fig. 3, a and b) also has no parallel in the pectin or AG M_r profiles. Pressure-probe measurements (8) show that, when pea internode segments are held in moist air, as in our air pretreatment, their turgor pressure decreases, by *in vivo* stress relaxation, to about half of full turgor within the time of our pretreatment period. Subsequent exposure to water allows nearly full turgor to redevelop rapidly (9). That this turgor step-up induces the XG M_r upshift is supported by the finding that segments whose turgor pressure is reduced by mannitol pretreatment also show an XG M_r upshift upon transfer to dilute buffer (Fig. 4). Turgor effects on metabolism of wall components have been previously reported (36, 45). However, a turgor step-up can cause rapid cell enlargement (19, 46), so the M_r upshift might be a response to this wall expansion rather than to turgor pressure itself. Because larger XG molecules should tend to make the wall stiffer or stronger, the M_r increase may constitute a feedback control that limits the amount of wall expansion and cell enlargement that can result from a turgor step-up. Kinetic experiments indeed indicate that the elevated cell enlargement rate induced by a turgor step-up quickly becomes suppressed (19, 46).

Significance of IAA Effects on XG M_r

IAA-induced cleavage of XG chains may weaken or loosen the wall leading to cell enlargement, as suggested in much previous literature (16, 17, 20, 41). A possibly analogous IAA-induced breakdown of hemicellulosic mixed-linked glucan has been considered a basis for wall loosening in grass coleoptiles (23, 35). We detected a large IAA-induced downshift in pea XG M_r within 30 min, in agreement with earlier findings that IAA induces a release of soluble XG from pea cell walls beginning within 15 min (34). This is consistent with a role in wall loosening, because cell enlargement in pea is stimulated after only 10 to 15 min of exposure to IAA (4, 28). A comparably early effect has not been demonstrated for the IAA-induced glucan breakdown in coleoptiles, whose significance in wall loosening has been challenged (21). However, Inouhe and Nevins (25) recently reported that antisera against the β-glucanases that are responsible for glucan breakdown inhibit not only this breakdown but also IAA-induced cell elongation in maize coleoptile segments, supporting the role of glucan breakdown in wall loosening. Similarly, Hoson and Masuda (24) reported that, in azuki bean segments, lectins directed against fucose inhibit (presumably by binding to the fucosyl groups of XG) both the IAA-induced downshift in M_r of XG and IAA-stimulated elongation. They considered this to demonstrate the role of IAA-induced XG breakdown in wall loosening. Unfortunately, the IAA-induced elongation rates given for the control segments in both studies just cited were extremely small compared to typical IAA-stimulated rates for these materials, so the amount of growth actually inhibited by the antibodies or lectins was only a small fraction of the normal growth performance of the cells, leaving the conclusions open to question.

Our results reveal no simple, general relation between XG M_r and elongation rate. Whereas IAA treatment of buffer-pretreated segments causes the M_r to quickly decrease, the XG M_r of air-pretreated segments remains almost steady for the first hour of exposure to IAA (while the control's M_r increases), although IAA stimulates elongation to the same degree in both types of segments. In air-pretreated pea segments, an IAA-induced actual M_r downshift (relative to initial M_r) appears only when the control's transient upshift has been completed and its XG M_r is returning to its initial value. Furthermore, XG M_r is not downshifted by low external pH nor by FC, even though these are known to cause a short-term elongation rate at least as great as IAA induces (40).

That XG M_r in air-pretreated segments remains unchanged during the first hour of exposure to IAA is apparently because the IAA effect tending to decrease the M_r is being superimposed on the turgor step-up effect tending to increase it. Similarly, the apparent lack of an XG M_r downshift in response to FC or low pH may be due to the M_r feedback response to rapid wall extension, suggested above to explain the turgor step effect: the rapid cell enlargement induced by FC or low pH could provoke a large M_r upshift that outweighs an effect of low wall pH *per se* tending to decrease the M_r. That both FC and low pH caused a modest upshift of XG M_r supports this explanation. A low pH-induced XG M_r decrease has been reported for other systems (26, 37, 43) and is suggested for pea by acidic buffer-induced release of XG from the cell wall (27). If XG M_r is in a dynamic state, as suggested above, XG chains could be broken (by the actions of IAA or low wall pH) to cause wall loosening at the same time as they are being extended (by the feedback response to rapid wall expansion), so the XG chain-breaking process would be undetectable, by the present method, under these circumstances. However, other processes may in addition, or instead, be providing an alternative means of wall loosening, *e.g.* introduction of new polymers into the wall, which is known to be stimulated by IAA in pea (1, 32, 47).

In contrast to bulk wall XG, the M_r of newly synthesized XG begins to shift down in response to IAA only after about 1 h (Fig. 8). This suggests that not until about 60 min after their deposition in the wall do new XG chains become subject to IAA-induced degradation. However, IAA causes a considerably larger eventual downshift in the M_r of newly deposited than of total wall XG (about sixfold *versus* two- to threefold, respectively). Furthermore, the M_r peak becomes so completely downshifted by 1.5 h (Fig. 8c) that the new XG that is being synthesized and deposited at the end of this period must be downshifted, like the previously deposited [^{3}H]XG. This could represent another biochemical factor in wall loosening: by exchange of noncovalent bonding relationships, the substitution of new, smaller XG chains for older, larger ones in the wall structure might well weaken or loosen it.

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